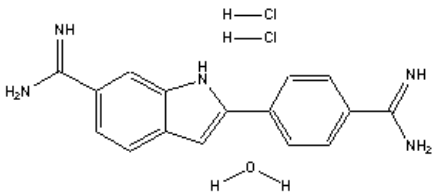
Catalog Number: 157574

4',6-Diamidino-2-phenylindole dihydrochloride

Structure:



Molecular Formula: $C_{16}H_{15}N_5 \cdot 2HCl$

Molecular Weight: 350.2

CAS # : 28718-90-3

Synonym: DAPI

Physical Appearance: Yellow to orange crystalline powder

Fluorescence:

DAPI: Excitation: 340 nm; Emission: 488 nm
DAPI-DNA Complex: Excitation: 364 nm; Emission: 454 nm (100 mM NaCl, 10 mM EDTA, 10 mM Tris, pH 7)[12]

Extinction Coefficient (263 nm): 30 (water)[7]

Solubility: Slightly soluble in water (10 mg/ml-clear, yellow to yellow-green solution - heat or sonication may be required). MP does not recommend the use of PBS or other neutral pH aqueous buffers for stock solutions because there may be solubility problems. At 1 mg/ml in water, solutions are stable in the dark at 2-8°C for approximately 2-3 weeks. For long term storage (3-4 months) it is recommended to aliquot the portions and store at -20°C. All solutions, no matter the storage temperature, should be stored in dark bottles, protected from light.

Description: DAPI is a cell permeable fluorescent minor groove-binding probe for DNA. It binds to double-stranded DNA by forming a stable complex which fluoresces about 20 times more than does free DAPI. Although divalent or heavy metal cations will quench DAPI's blue fluorescence, the fluorescence is unchanged over a pH range of 4-11. The DAPI-DNA complex is stable for a couple hours at room temperature and is not photo-dissociated during assay.[1] DAPI is several times more sensitive than ethidium bromide for staining DNA in agarose gels.

DAPI stains DNA specifically and quantitatively. DAPI selectively forms complexes with A-T, A-U and I-C base pairs (preferably A-T rich DNA), having one molecule of dye for every 3 base-pairs.[12] The fluorescence is directly proportional to the amount of DNA present. For fluorimetric titration of the DAPI-DNA complex, the excitation maximum is 364 nm, emission maximum is 454 nm (100 mM NaCl, 10 mM EDTA, 10 mM Tris, pH 7).[12] DAPI in aqueous solution has two absorbance maxima, at 349 nm and at 263 nm.[7]

For axonal trace studies, DAPI was mixed with primuline (approximately 0.5 ul of 2.5% DAPI, 10% primuline (w/v) in distilled water) and injected into rat brain. This mixture fluoresces blue with gold granules.[12]

DAPI can be used as a vital dye to stain nuclei in mature living pollen grains in conjuction with fluroescein diacetate (used to indicate viability of pollen, etc.).[4] For vital staining, 1 ug/ml in cell growth medium was used; for post-vital staining, materials were stained with 0.1-0.5 ug/ml in water.[3] Usage concentration in pollen germination medium ranges from 0.25 to 5.0 ug/ml. Prior to staining, fixed materials were immersed briefly in 200 mM KCl, then stained with 0.25 ug/ml DAPI (in buffer) for one hour. Without rinsing, the samples could be covered with cover slips (sealed with nail polish) and examined up to 24 hours later if stored at 2-8°C in the dark.[4]

DAPI binds differently and highly specifically to yeast mitochondrial and nuclear DNA. These complexes are useful in detection of mycoplasmas. In uninfected cell culture, DAPI is rapidly taken up into cellular DNA, yielding highly fluorescent nuclei with no detectable cytoplasmic fluorescence. Cultured cells are examined immediately after incubation with DAPI at 0.1 ug/ml in PBS at 30°C for 15-30 minutes.[3] If cells are contaminated with mycoplasma, characteristic discrete fluorescent foci are readily detected in cytoplasm and on cell surfaces.[3,19] The presence of these foci is a convenient diagnostic test for contamination.

Typical Mycoplasma Detection Protocol[2,8,16]

Required Materials:

A. Indicator cells, Vero (ATCC CCL 81) or 3T6-Swiss albino (ATCC CL 96)
B. Leighton tubes or glass cover slips/culture dishes
C. Cell culture medium (growth medium)
D. Methanol
E. Glacial acetic acid
F. DAPI
G. Mounting Solution McIlvaine's Buffer: Glycerol [1:1]
H. Fluorescent microscope (see examining cultures)
I. Caronoy's fixative: prepare fresh for each use. To prepare use 3 parts of the methanol to 1 part of the glacial acetic acid.

Culturing Samples and Indicator Cells:

1. Seed indicator cells at low density in a Leighton tube or on a glass coverslip in a culture dish containing tissue culture medium. Incubate for 24 hours at the conditions appropriate for the culture medium (typically 37°C at 2-5% CO_2). Prepare enough cultures to inoculate with control and test samples.
2. To separate indicator cell cultures, add 0.1 ml of test samples.

    a. Negative control: Indicator cell cultures inoculated with 0.1 ml of culture medium.
    b. Positive control: If a positive control is desired, infect a culture of the indicator cells with 0.1 ml of a viable culture of mycoplasma species.

3. Allow all cultures to incubate for an additional 4 days.

NOTE: It is important to stain and examine cultures before they reach confluency. Adjust incubation time and inoculum density according to the growth characteristics of the test and indicator cells.

Fixing Cells:

1. Prepare Carnoy's fixative fresh on the day of use. Prepare enough solution to fix all cultures. Approximately 15 ml of fixative is required per culture.
2. Without decanting growth medium, add approximately 5 ml of Carnoy's fixative to each culture and allow to stand 5 minutes.
3. Decant and add 5 ml of fixative to the cultures and allow to stand 5 minutes.
4. Decant fixative, add 5 ml of fresh fixative, and allow to stand 5 minutes.
5. Finally, decant fixative and allow growth surface to air dry approximately 5 minutes.

Staining and Mounting Cells

1. Prepare working concentration of DAPI stain by solubilizing DAPI in water at 0.1 mg/ml.
2. Completely immerse the growth surface in the stain solution and allow to stand for 15-30 minutes.
3. Rinse twice with distilled water.
4. Mount growth surface, cell side down, with a drop of mounting solution on a microscope slide. Slides may be preserved by sealing the edges of the cover lsip and slide with clear nail polish. Slides should be protected from light and heat. These will last several weeks without quenching if properly stored.

Examining Cultures:

A fluorescent microscope capable of epifluorescence is needed for visualizing the stain preparations. A typical system includes fluorescent microscope with a 53/44 barrier filter and a BG-3 exciter filter. A total magnification of 500X (40X; 12.5) is usually sufficient to visualize mycoplasma but higher magnification may be used.

References:

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